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Research Article

Unraveling the microbiome dynamics of the invasive *Acacia longifolia*: a closer look at seeds and nodules

Joana G. Jesus[1,2], Andreia Anjos[1,2], Cristina Máguas[1,2], Helena Trindade[1,2]

1 *Centre for Ecology, Evolution and Environmental Change (CE3C), Faculty of Sciences of the University of Lisbon (FCUL) & Global Change and Sustainability Institute (CHANGE), Lisbon, Portugal*
2 *Faculty of Sciences of the University of Lisbon (FCUL), Lisbon, Portugal*
Corresponding author: Joana G. Jesus (jgjesus@fc.ul.pt)

Abstract

Acacia longifolia, a species native to Australia, is an aggressive invasive in Mediterranean-type ecosystems worldwide. Its success in diverse habitats, expanding from coastal dunes to forests, is often attributed to its ability to establish interactions with a variety of microbes, including bacteria and fungi. This study investigates the seed and root-nodule microbiomes of *A. longifolia* to understand the roles these microbial communities play in its adaptation and invasive behaviour. Using high-throughput sequencing, we characterized the bacteriome and mycobiome associated with the plant, considering nodules and seeds, and the surrounding soil in three different locations in Portugal with different climate conditions (North, Center and South), and a comparison between two different habitats (Dune *versus* Forest). Our results reveal a dynamic interaction between *A. longifolia* and its microbial partners, supporting the importance of these plant-microbe interactions in nutrient acquisition and stress tolerance for *A. longifolia*, ultimately leading to their impact in an invaded ecosystem. The seed microbiome of *A. longifolia* was less diverse than for nodules but with more functions assigned, while nodules showed a broader diversity, assigned to more specific functions. Here we provide evidence for the role of seed microbiota in germination and seed-to-seedling transition along with the beneficial role of nodulation in development and seedling-to-sapling switch. We also propose a local signature for microbial communities as we found a dissimilarity in microbial partners when considering habitat, with dune communities showing a functional plasticity, aiding *A. longifolia* to cope in such nutrient-limiting environment. For forests, functions more related with plant and microbe associations are evidenced, possibly facilitating interspecific competition. These findings contribute to an understanding of the plant-microbe interactions and dynamics that underpin *A. longifolia* ecological success as an invasive plant.

Key words: Dissimilarity, functional prediction, habitat adaptation, microbial communities

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Introduction

Invasive plant species are a major driver of biodiversity loss and ecosystem degradation worldwide, being responsible for disrupting native plant communities and altering soil properties (Millennium Ecosystem Assessment 2005; Pyšek et al. 2012; Dostál 2024). Among such species, *Acacia longifolia* Andrews (Willd.), a leguminous shrub native to Australia, has become highly invasive in Mediterranean-type ecosystems, mainly in coastal dunes where it was introduced to prevent erosion (Peperkorn et al. 2005). In Portugal, *A. longifolia* is no longer restricted to

coastal dune ecosystems, extending to native and non-native forests from the north to the south of Portugal (Colaço et al. 2023). This spread raises the question of what adaptive traits this species possesses that allow its success.

Several factors are responsible for the spread of *Acacia* spp., including the production of a high number of seeds, high seed longevity, and increased seed germination after fire events, just to mention a few (e.g., Richardson and Kluge 2008; Palmer 2016; Riveiro et al. 2020). Being an invasive species, a key factor that could trigger its success is the microbiome (Lau and Suwa 2016). Following an introduction, particularly in Fabaceae-belonging species, studies raised the hypothesis of these plants' inadaptation, considering the absence of compatible rhizobia (i.e., nitrogen-fixing bacteria) (Simonsen et al. 2017). However, in the case of *Acacia* spp., their promiscuity can help in finding suitable partners within soil microorganisms in the novel habitat (Birnbaum et al. 2012; Klock et al. 2015; Jesus et al. 2020). Some studies already show evidence for a co-introduction of rhizobial partners (Rodríguez-Echeverría 2010; Crisóstomo et al. 2013) or the possibility of novel symbiotic relations (e.g., Ndlovu et al. 2013; Jesus et al. 2020, 2023). The ability of *A. longifolia* to fix nitrogen, alongside with its rapid growth and dispersal, enables it to outcompete native vegetation. This causes significant changes in ecosystem structure and function, with *Acacia* spp. playing a role as ecosystem-engineers (Stock et al. 1995; Marchante et al. 2004, 2008, 2009; Yelenik et al. 2007). This includes high water consumption (Marchante et al. 2003), nutrient cycles' alterations (Ulm et al. 2017; Meira-Neto et al. 2018) and increased biomass production (Ulm et al. 2019, 2022).

Soil is considered essential to ecosystem functioning hosting a high biodiversity (Delgado-Baquerizo et al. 2016; Schuldt et al. 2018; Liu et al. 2023) impacting soil vitality, which is mostly dependent on microbial communities' activity and interactions (Johns 2017). This is critical in invaded areas, where invasive plants significantly alter soil communities to establish and spread further, while simultaneously causing profound changes that constrain native plant diversity (e.g., Lau and Suwa 2016). Plant-associated microorganisms play crucial roles in plant development, growth and plasticity (Bulgarelli et al. 2013; Imam et al. 2016; Jain et al. 2024) and, particularly, seed-associated microbes are crucial for germination and early-establishment (Fukami 2015; Bergmann and Leveau 2022). Seed microbiota can include endophytes and epiphytes. While endophytes reside inside seed tissues and are vertical- or horizontally transmitted, epiphytes are colonizers of seed surface and may not become part of the seed microbiome (Barret et al. 2015). As it was proposed by several studies (e.g., Malfanova et al. 2013; Truyens et al. 2015), seed microbiota can be a hotspot of beneficial bacteria and fungi, making this microbiota an important player on seed-to-seedling transition (Fenner and Thompson 2005; Leck et al. 2008). Also, previous studies on *A. longifolia* showed that in its invasive range, this species has a combination of endophytes that include plant growth promoting bacteria, providing extra capabilities that helps growth and expansion (Condessa et al. 2024). Furthermore, root-nodules house several growth promoters and provide a direct source of nitrogen, crucial in the seedling-to-sapling transition (Desbrosses and Stougaard 2011; Pucciariello et al. 2019). From soil-to-seed and from soil-to-nodule, studies have been raised hypothesizing a dynamism that could determine plant adaptation, and these transitions can be local- and individual-dependent, respectively (Nelson 2018; Wang and Zhang 2023). The diversity and dynamics of microbial communities associated with seeds and

root-nodules require further investigation in this aggressive invasive plant, as they may hypothetically trigger new dispersal events and serve as a lever for successful adaptation, facilitating drastic environmental changes.

Accordingly, this study aims to (i) explore how seed and root-nodule microbiomes vary across different habitats and edaphoclimatic conditions, (ii) identify key species within the bacteriome and mycobiome of seeds and root-nodules at a local scale and (iii) determine the potential functional roles played by these microbial communities. These findings could elucidate how microbial communities support the invasiveness of *A. longifolia*, ultimately contributing to mitigate its impacts on native biodiversity and ecosystem functioning.

Material and methods

Field sampling

Sampling was performed in spring 2023 (i.e., during March) in three locations with distinct climate conditions in Portugal's mainland: Mira (hereafter referred as "North" and described as "Very Humid"), Fonte da Telha ("Center" and "Humid") and Vila Nova de Mil Fontes ("South" and "Semi-Humid"), respectively. Classification of these locations was performed according to De Martonne aridity index (De Martonne 1925). At each location, two habitats invaded by *Acacia longifolia* were selected, a Dune and a Forest, in which ten *A. longifolia* young saplings were selected (ca. 60 ± 13 cm in height), with saplings separated by at least 10 m from each other (to cover microsite variability). Each sapling was carefully dug up to collect roots with attached nodules, which were stored in silica gel to dehydrate until further processing. Ten soil samples (10–20 cm in depth) were collected close to each of the saplings (i.e., one sample per sapling) and stored in a freezer (-80 °C) for microbial assessment. Mature pods were collected directly from the branches of ten adult *A. longifolia* trees, with each tree again selected based on proximity to the collected saplings. Seeds were then separated from pods and stored in paper bags at room temperature pending further analysis. All the material used for sample collection was disinfected with ethanol among saplings in each habitat and location.

An overview of each location and their climatic conditions including temperature and precipitation in the six months before sampling date are presented in Suppl. material 1: fig. S1. Also, soil physicochemical parameters were assessed to characterize each location, and this data is presented in Suppl. material 1: table S1. Soil analysis were performed in Laboratório de Plantas e Solos da Universidade de Trás-os-Montes e Alto Douro (UTAD), Portugal. A vegetation survey was conducted using a 60 × 60 cm quadrat nearby each sapling to understand other plant species presence and cover. Most of the species were herbaceous whose influence is mostly negligible.

Sample processing

The three biological samples were prepared for high-throughput sequencing: root-nodules, hereafter named nodules, seeds and soils in composite samples, ending up with 18 samples to represent each biological sample from each habitat in each location.

Next-generation sequencing (NGS) through Oxford Nanopore © was performed to infer microbial communities of nodules, seeds and soil from each habitat in each location. Both nodules and seeds were surface disinfected before

processing. For pooled seeds (n = 3 from each adult tree) and pooled nodules (n = 5 from each sapling) samples, total DNA was extracted using the modified CTAB protocol (Yuan 2019). Regarding soil, total DNA was extracted from 10 g of each soil using the DNeasy PowerMax Soil Kit from Qiagen, following the manufacturer's instructions. DNA purity was assessed by absorbance at 260 nm using a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, USA). The extracted DNA was stored at -20 °C pending further processing. For all samples, 12.5 μL of eluted DNA obtained in the extraction protocol was used for library preparation. Sequencing runs were performed using R10.4.1 flow cells on a PromethION sequencing platform and sequencing data were acquired in real time using MinKNOW 18.08.2 software. Sequencing data was stored in fastq files, with each file representing a batch of 4000 reads.

Sequencing data was derived from 16S or 25S - 28S amplicons (for Bacteria and Fungi, respectively), with the exclusion of low-quality reads. The remaining reads underwent size selection, retaining those with lengths between 1200 bps and 1700 bps, accomplished through prinseq-lite (Schmieder and Edwards 2011). Taxonomic classification employed a Lowest Common Ancestor approach, utilizing an index based on k-mers that map to the lowest common ancestor of all genomes known to encompass a specific k-mer (Wood et al. 2019).

Statistical analysis

The composition of bacterial and fungal communities was used to generate heatmaps based on the abundance data of the top 10 genera identified in each sample. Abundance data were transformed using z-score normalization to enhance the comparability across samples. Samples (rows) and Operational Taxonomic Units (OTUs, columns) were clustered hierarchically based on relative abundance profiles, using the Bray-Curtis distance and the Ward's method as clustering algorithm.

All OTUs, for both bacteriome and mycobiome, were used to calculate Shannon-Wiener (Shannon 1948) and Chao (richness) (Chao 1984) indexes considering biological sample x habitat x location (i.e., 18 composite samples tested). To explore differences in microbial communities among samples, a Principal Coordinates Analysis (PCoA) was performed using the Bray-Curtis dissimilarity index to construct the distance matrix. The first two principal coordinates, which explain most of the variance in the data, were visualized to highlight clustering patterns among samples, which statistical significance was tested using PERMANOVA (Permutational Multivariate Analysis of Variance).

Functional predictions for bacterial and fungal communities were performed using all classified taxa from each biological sample, applying FAPROTAX v1.2.6 (Louca et al. 2016) for bacteria and FungalTraits (Põlme et al. 2020) for fungi. Presence and absence matrixes were built according to these databases and a cumulative relative abundance for each identified function was calculated. Ternary plots were generated to visually represent the relative abundances of key functional groups assigned to the different OTUs identified in both microbial communities (see Suppl. material 1: tables S2, S3).

Data analyses were performed using the packages *betapart* (Baselga et al. 2023), *ggtern* (Hamilton and Ferry 2018), *microeco* (Liu et al. 2021), *pheatmap* (Kolde 2019), *rstatix* (Kassambara 2023), *stats* and *vegan* (Oksanen et al. 2022) in R (v.4.2.2) (R Core Team 2023).

Results

Regarding the top 10 genera of bacterial communities, they were grouped in two main clusters, one including mostly soils (Cluster I) and the other comprising seeds and nodules' communities (Cluster II) (Fig. 1). The cluster that contained most of the seed samples (Cluster III) included genera that were not the most abundant in soil, mostly including the phylum Cyanobacteriota, and the genera *Synechococcus*, *Oscillatoria*, *Nostoc* and *Stanieria.* For nodules, assigned to Cluster IV, *Bradyrhizobium* was a representative genus along with *Bacillus*, *Paenibacillus* and *Stenotrophomonas.* In soils, *Paraburkholderia*, *Conexibacter*, *Mucilaginibacter*, *Paludibaculum* and *Candidatus* Koribacter were more dominant among samples (Cluster V). There was no clear clustering based on location, however, dispersed clusters were formed formed considering habitat, especially for soil, in Cluster I, with Dune samples presented together as well as Forest samples.

Regarding the top 10 genera for fungal communities, three main clusters grouped biological samples: Cluster I for nodules, Cluster II for seeds and Cluster III for soil (Fig. 2). Considering the communities found, there were two main clusters, one that included the representative genera (i.e., more abundant) in soils (Cluster IV) and another for nodules and seeds (Cluster V). *Amanita*, *Russula*, *Saitozyma*, *Fusarium* and *Acremonium* were among the more abundant genera for Cluster IV; included in seed mycobiome, *Alternaria*, *Aureobasidium*, *Entoloma*, *Cladosporium* and *Curvularia* were identified, while for nodules, *Solicoccozyma*, *Umbelopsis*, *Serendipita* and *Tuber* were among the most abundant. In this case, there was a more evident distinction when considering habitat, especially for seeds (i.e., higher z-score value of 2 to 4).

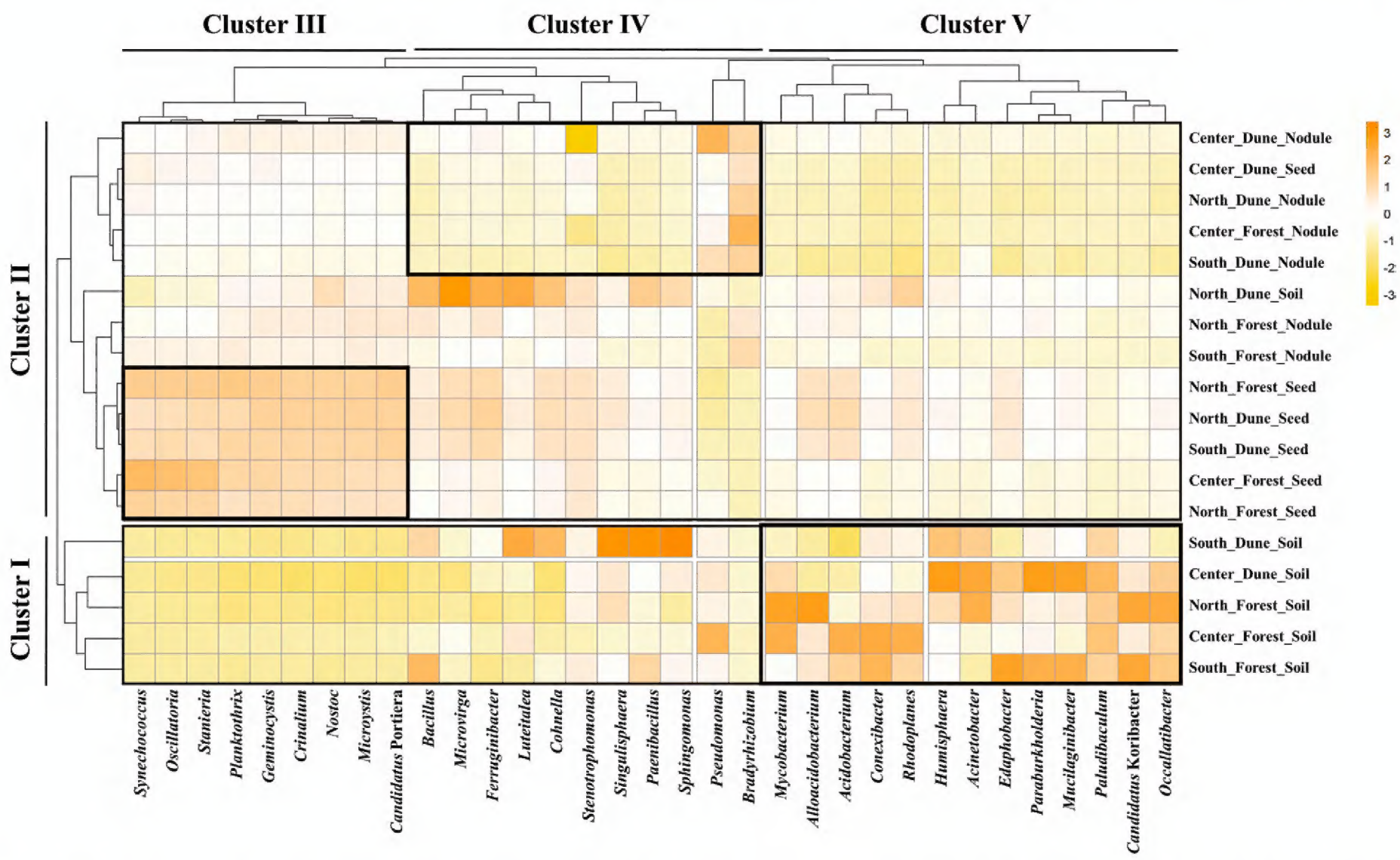

Figure 1. Heatmap based on bacterial communities at genus level (top 10) considering biological samples (nodule, seed and soil), habitat (dune and forest) and location (North, Center and South). Samples (rows) and Operational Taxonomic Units - OTUs (columns) were clustered hierarchically based on relative abundance profiles. These values were standardized in z-scores (varying between -3 and 3, against each mean value). Sample names stand accordingly: Location_Habitat_Biological sample.

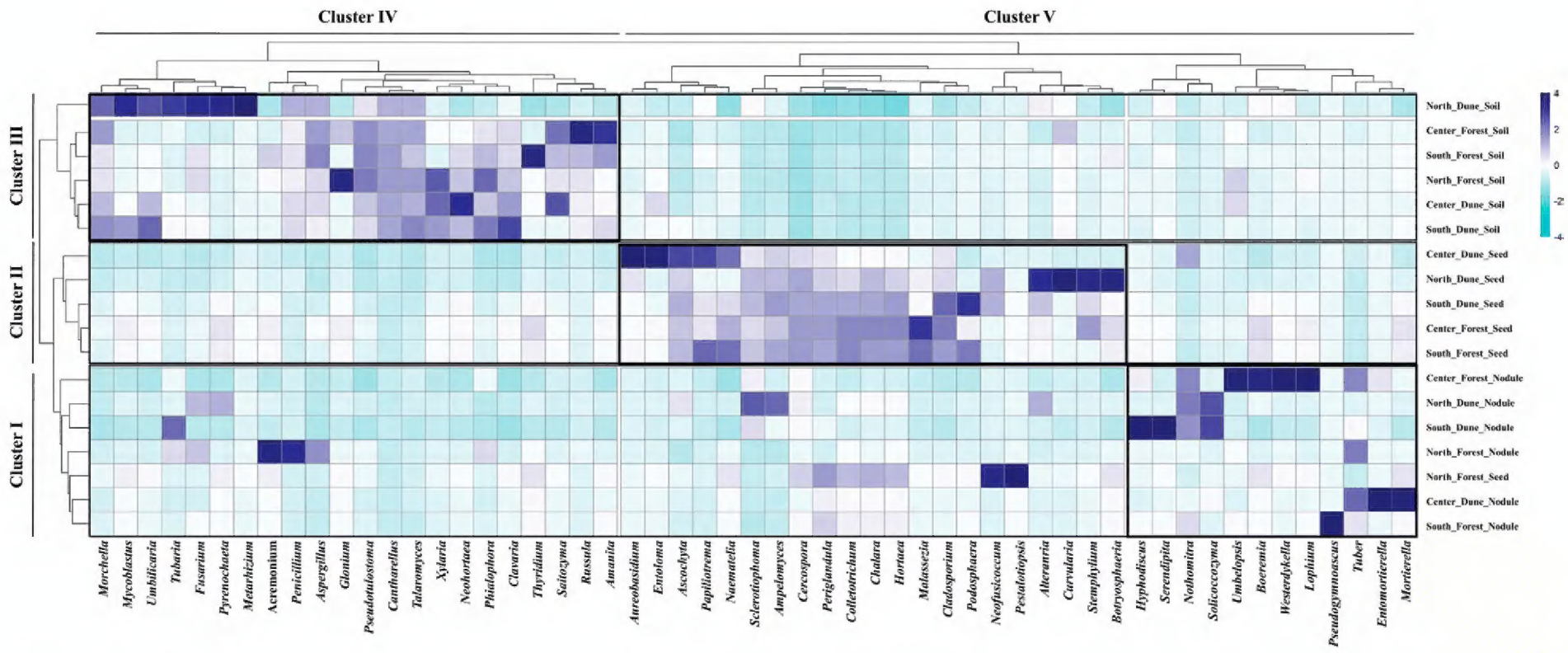

Figure 2. Heatmap based on fungal communities at genus level (top 10) considering biological samples (nodule, seed and soil), habitat (dune and forest) and location (North, Center and South). Samples (rows) and Operational Taxonomic Units - OTUs (columns) were clustered hierarchically based on relative abundance profiles. The values were standardized in z-scores (varying between -4 and 4, against each mean value). Sample names stand accordingly: Location_Habitat_Biological sample.

For both bacterial and fungal communities, dissimilarity analysis highlighted the clustering by biological sample, regardless of both habitat and location (Fig. 3, Suppl. material 1: fig. S2). Within the bacterial communities, there was greater dissimilarity between soils and nodules or seeds, resulting in an overlap between the latter two. Overall, bacterial communities were more dissimilar within nodules than when compared with seeds. In fungal communities, there was a greater similarity among different samples within each biological sample, and a higher dissimilarity between the three biological samples, regardless of habitat and location.

Soil samples had the highest number of OTUs with a greater abundance of bacteria compared to fungi. In contrast, seeds and nodules showed a higher prevalence

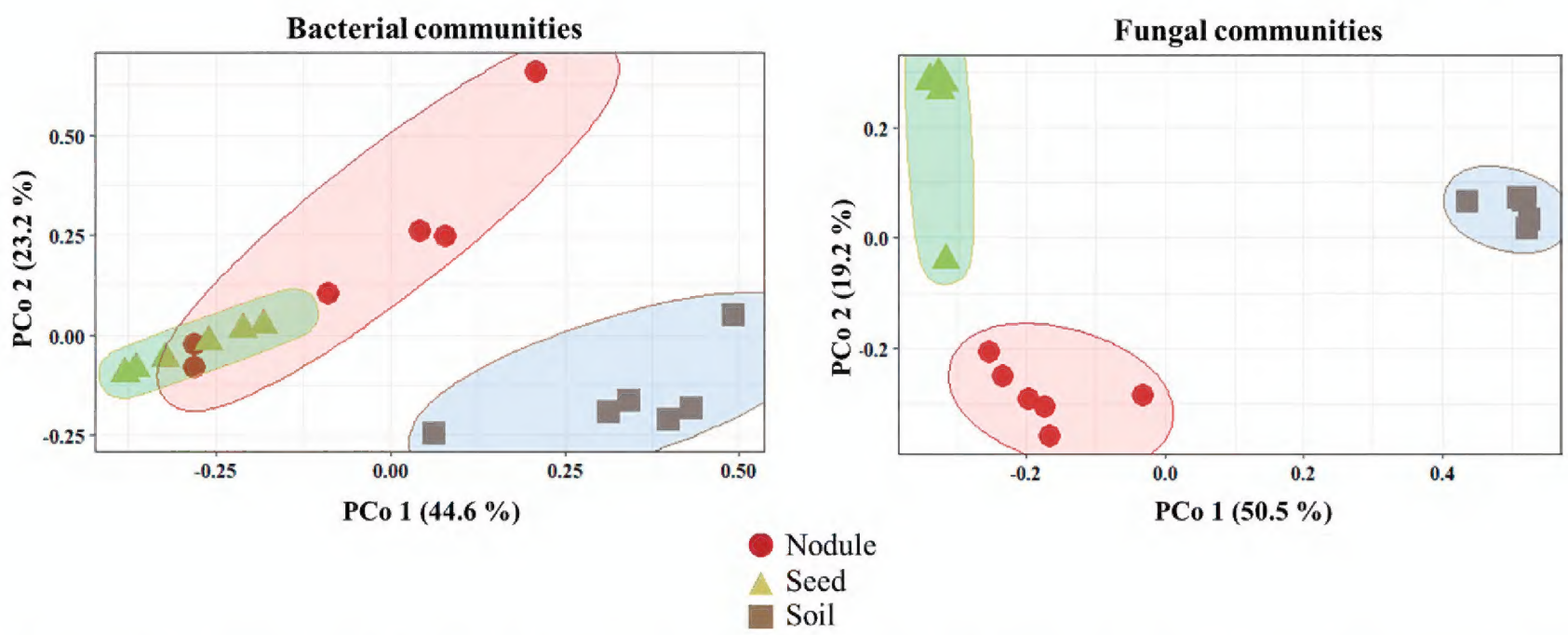

Figure 3. Principal component analysis (PCoA) based on dissimilarity matrixes with Bray-Curtis distance method, using classified Operational Taxonomic Units (OTUs) for bacterial and fungal communities, considering the three biological samples (nodules, seeds and soil), each habitat (dune and forest) and the three locations (North, Center and South). Colors and shapes were assigned to each biological sample: red circles for nodules, cream triangles for seeds and light brown squares for soil. Ellipses were used to highlight the clustering of each biological sample [(for bacterial communities: pseudo-F = 10.05, p = 0.001, 999 permutations), with biological sample explaining 57.3% of the variance in community composition (R^2 = 0.573); for fungal communities: pseudo-F = 15.21, p = 0.001, 999 permutations), with biological sample explaining 66.9% of the variance in community composition (R^2 = 0.669)]. Note that y-axis has different scales in each community.

of fungi than bacteria, with seeds displaying the lowest overall OTU counts for bacteria (Suppl. material 1: table S4).

In both microbial communities, while biological sample and habitat had an influence on diversity and richness, location did not influence significantly. Also, soil exhibited the highest diversity and richness comparing with nodules and seeds. For bacterial communities, nodules were more diverse and showed richer communities than seeds. Overall, dune samples had higher diversity and richness than forest samples (Fig. 4a). In contrast, for fungal communities, seeds had higher values of diversity and richness than nodules and showed greater variability among samples. A similar pattern of higher dispersion of diversity was observed across habitats, with dune samples again displaying higher values (Fig. 4b).

For functional characterization, considering bacterial communities, the most common function in nodules was assigned to Nitrogen metabolism, mostly nitrogen fixation, higher in samples from dunes (88%) compared to forests (ca. 44%) (Fig. 5a, Suppl. material 1: table S5). General metabolism related functions were also present in nodules, accounting for 60% for methylotrophy in dunes, and only 25% in forest samples. Also, when considering Defense, including aromatic

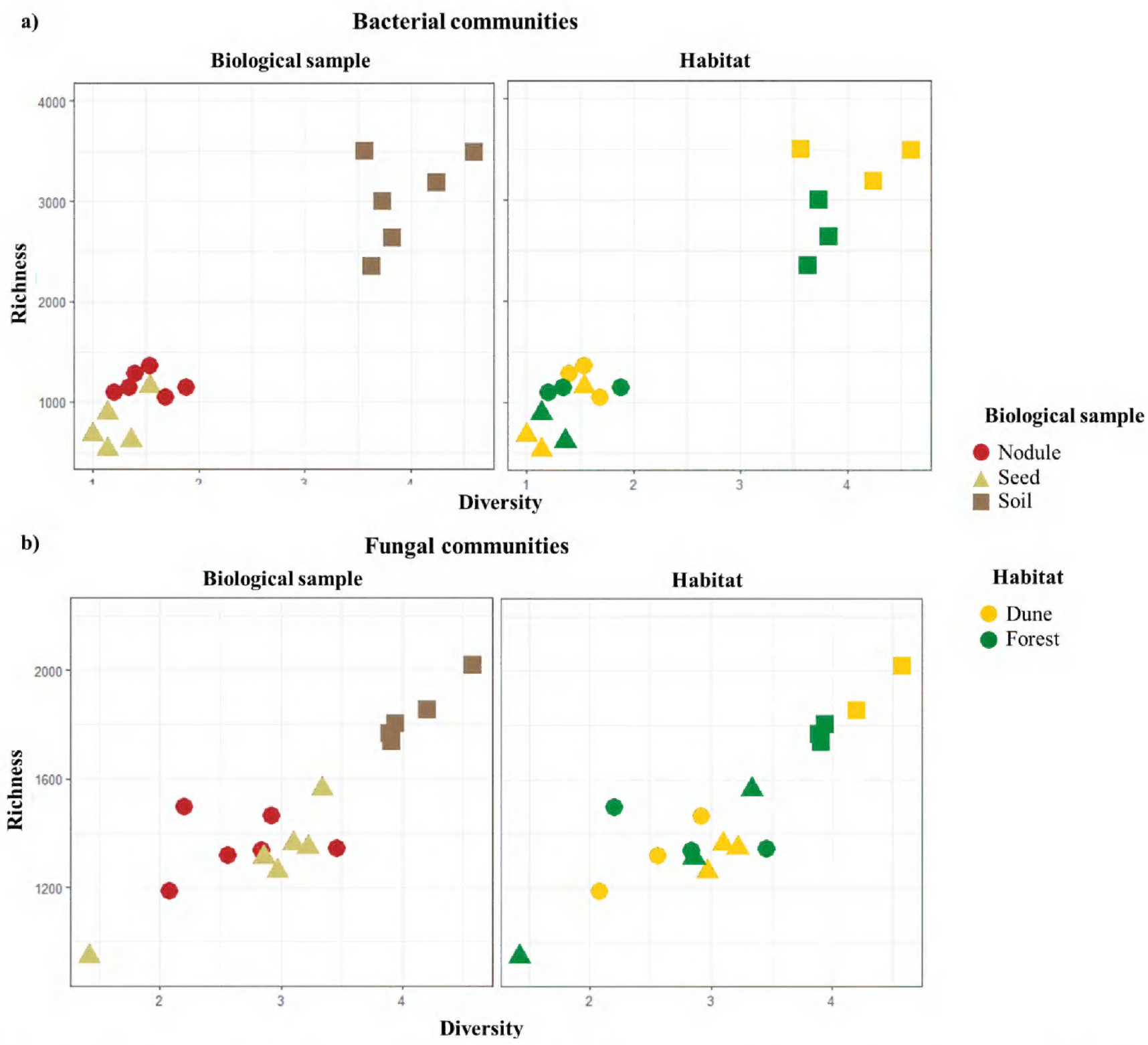

Figure 4. Diversity (Shannon-Wiener index) and Richness (Chao index) considering **a** bacterial and **b** fungal communities, for biological samples (nodule, seed and soil), habitat (dune and forest), regardless of location (North, Center and South). Note that y-axis has different scales in each community.

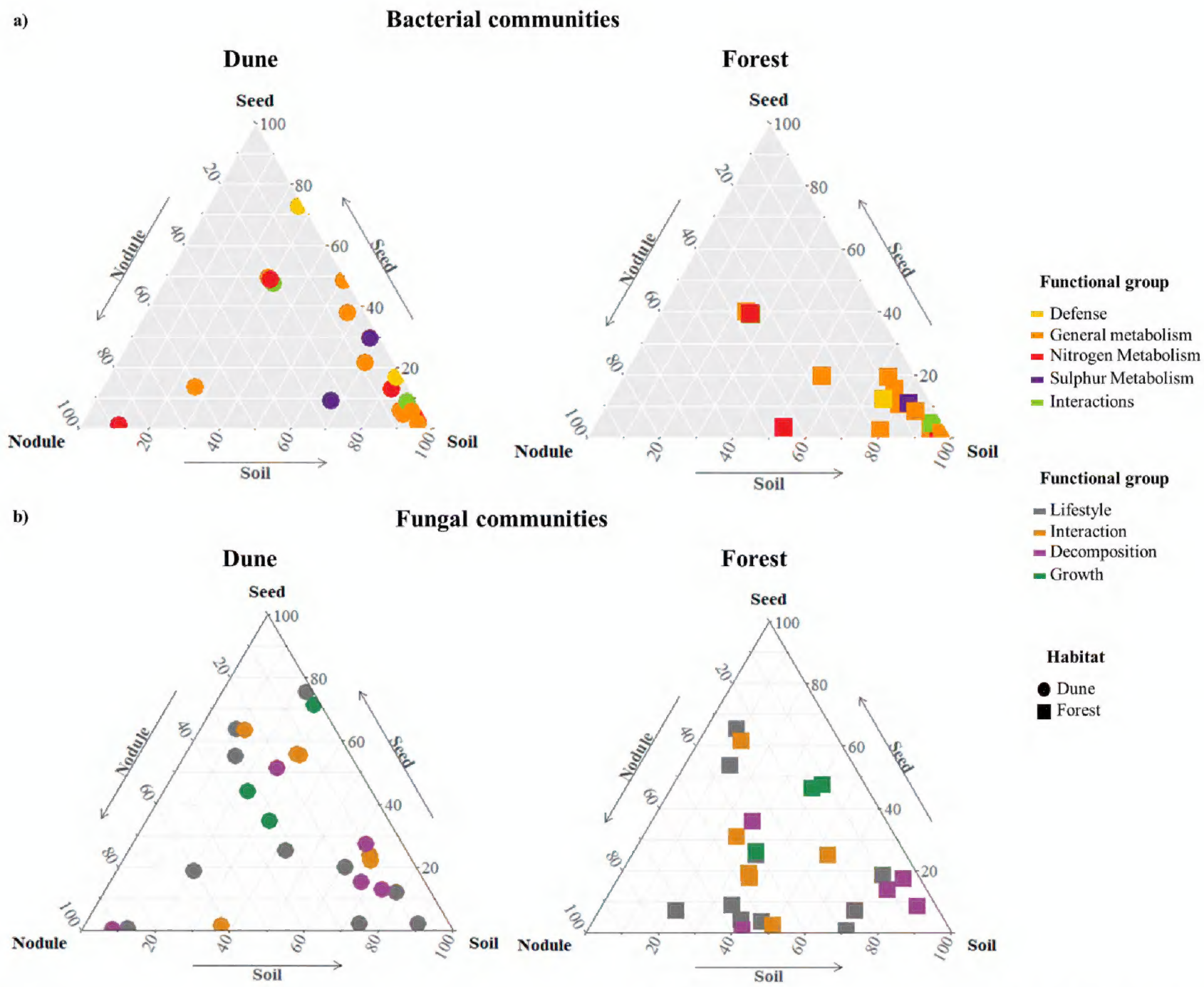

Figure 5. Ternary plots presenting functional predictions for a) bacterial and b) fungal communities using FAPROTAX v1.2.6 (Louca et al. 2016) and FungalTraits (Põlme et al. 2020), respectively. Each function abundance (mean values considering the three locations, North, Center and South) is presented for each biological sample (nodules, seeds and soil) and for each habitat (dune and forest). Colors are assigned to functional groups identified and shapes to habitat.

compounds degradation, ca. 36% were found in forest samples compared to 22% for dunes. In dunes, Sulfur metabolism related functions were also registered, accounting for 24% of sulfur compound degradation.

Seeds gathered a greater variety of functions, and all functional groups identified were included in dune habitat. A greater abundance of different General metabolism related functions (e.g., chitinolysis, ligninolysis and manganese oxidation), Nitrogen-related metabolism (mainly nitrogen respiration), Sulfur metabolism (i.e., dark oxidation of sulfur compounds) and Defense, as aromatic compound degradation was found. Considering forests, the potential functional pool was mostly present in soils.

For fungal communities, there was a more evident distinction between habitats, having Interaction as the main functional group associated with forest, and dune relied more on Growth and Decomposition (Fig. 5b, Suppl. material 1: table S6). Nodules from forest samples had functional machinery more abundant in Ecological Strategies (i.e., Lifestyle) and Interaction-related functions, including mycorrhiza and endophytes, particularly ectomycorrhizal (ca. 29%) and endophytic capacity *via* root and leaves (ca. 47%, each), respectively. Dunes had the same functional groups but more abundantly, highlighting the plant pathogens (64%) and soil saprotrophs (60%) alongside endophytic capacity *via* roots (62%).

Discussion

Acacia longifolia nodules and seeds showed distinct microbial compositions suggesting potential functions tailored to their specific roles in plant's lifecycle and ecological strategies. Indeed, we observed for the first time *A. longifolia* seed microbiome that seems to be the determinant for plant establishment despite its low diversity. On the other hand, nodules seem to be important for plant development, reflecting a more targeted functional machinery, especially in nutrient-poor environments as found in dune ecosystems. Altogether, this study points out the adaptative capacity of *A. longifolia* to interact with soil communities and cope with surrounding conditions, and its limitations.

Among bacterial communities, the phyla Pseudomonadota, Actinomycetota, Bacillota and Bacteroidota have been more described for both seeds and nodules microbiomes, where they are considered important, rendering its high abundance in soils (Fierer et al. 2012; Barret et al. 2015; Etesami 2022 and references within). In our study, alongside with these phyla, we identified a considerable presence of Cyanobacteriota inside both structures.

A more similar microbiome was found among seed samples slightly varying in abundance. Previous studies suggest a vertical transference from mother-plant (Johnston-Monje and Raizada 2011; Links et al. 2014), corroborating the divergence on seed compared to soil communities. This is evidenced by the lower abundance of the Cyanobacteriota in soils that gain representation in seed bacteriome. However, this more homogeneous microbiome provides a diverse set of functions that could be essential for germination and early establishment, such as variable metabolic pathways or defense strategies. The higher abundance of genera from Cyanobacteriota could be explained through their recently described mixotrophy (Muñoz-Marín et al. 2020), which renders resilience to the host plant. Host selection can ensure the success of establishment, which is also corroborated by the diverse functions assigned to carbon, nitrogen and sulfur cycles, especially in dunes, making seed microbiota highly plastic and efficient regardless of lower diversity.

Additionally, nodules also have a higher abundance of Cyanobacteriota corroborating previous results from our group (Jesus et al. 2023). It can be suggested that these bacteria are available *via* seeds, considering their low abundance in soils, highlighting a selection from host plant for stable partners within plant microbiome. *Bradyrhizobium* was the main genus associated with these structures, along with other Rhizobia as extensively described (e.g., Rodríguez-Echeverría 2010; Crisóstomo et al. 2013; Barret et al. 2015; Jesus et al. 2020), intimately associated with nitrogen fixation in both habitats. However, considering the habitat conditions, differences were observed, that balanced nutrient pools with energy demand, acknowledging nodulation as a costly process. On the other hand, in forests, it is less abundant, and these soils show a higher diversity of functions associated with the identified microbiome. This suggests that *A. longifolia* can be able to recruit a set of microorganisms more capable of triggering interactions, especially regarding fungal communities. This environmental shift allows this species to be more competitive and highlight nodulation as a process that enhances plant development. Indeed, while seedlings are growing to saplings, there is an essential carbon and nitrogen dynamics required (Zheng 2009), which can be facilitated by a more specialized microbiome. Furthermore, methylotrophy is more abundant in dunes; this process is essential in carbon cycling and plant growth, particularly

in nutrient-poor environments, where single-carbon compounds are more readily available than multi-carbon ones (as revised by Elbasiouny et al. 2022).

Apart from these more specific communities, *Pseudomonas* and *Stenotrophomas* were consistently present across all samples, regardless of habitat and location, being present in soil, seeds, and nodules. This corroborates previous studies that highlight the contribution of beneficial surrounding microbial communities to plant microbiome, as well as, if a microorganism is dominant in soil, it increases the probability of becoming dominant inside the plant (e.g., Bulgarelli et al. 2013; Lebeis et al. 2015; Flores-Duarte et al. 2022; Kumar et al. 2023). Environmental reservoir, niche specialization and priority effects determine this, which explains the differences in abundance, accounting for nodules and seed inherent filtering/selection processes. It is known that microbial lifestyle, i.e., being mutualistic or pathogenic, is clearly expressed in specific contexts. The specificity in which this (dis)advantage is explored considers both plant and microbe when in association, as suggested by Nelson (2018). This is relevant given the higher abundance of potential plant pathogens in seeds. Given that microbial functional redundancy is highly present in soils, the presence of a microorganism does not necessarily equate to it performing a specific function. On the contrary, we found less segregation between seed and nodules communities, but a higher divergence in potential functions. So, the promiscuity associated with a great adaptative behavior of *A. longifolia* makes it a highly plastic species.

Regarding mycobiome and the acquisition of fungal partners to the seeds, local assembly has been proposed as more relevant than host selection (Klaedtke et al. 2016); the main important phyla are Ascomycota followed by Basidiomycota (Links et al. 2014). In this study, which includes genera from both phyla, the community found seems to behave as endophytic, given its dissimilarity from soil communities, suggesting its heritage from mother plant. This includes the genera *Entoloma*, *Cladosporium* and *Curvularia* found in seeds. In nodules, *Solicoccozyma* was found, being also described as an endophytic fungus (Sarabia et al. 2018), having a role in nutrient cycling and being described as tolerant to a range of environmental conditions. Regarding dunes, *Umbelopsis*, *Serendipita* and *Tuber* were among the main genera and their roles in decomposition (Janicki et al. 2022), as beneficial root-associated fungus (Mahdi et al. 2022) and ectomycorrhizal (Monaco et al. 2022), respectively, could potentiate survival under more nutrient-limiting conditions. The simultaneous presence of these genera in seeds and root-nodules suggest a seed-to-nodule transmission (or vice-versa), and this intimate integration could trigger new specific interactions as proposed by Nelson (2018). Future studies should be focused on in-depth transcriptomics aiming to fully understand these communities and understanding in detail the transformative role of *A. longifolia* as an invasive plant in soils.

In this study, we found differences in microbial composition between the nodules and seeds *per se*, but further changes in the potential functional "status" can increase those differences, as suggested previously by Degens et al. (2000). The importance of metabolic plasticity, i.e., variety of functions, for some of the microorganisms is potentially raised here, hypothesizing two distinct roles for nodule and seed microbial communities. Nodule communities depend less on carbon acquisition, since the host plant provides the carbohydrates needed in exchange for fixed nitrogen; while for seeds, this plasticity can be important given the already known role of seed microbiome in germination and

early establishment (Fukami 2015; Bergmann and Leveau 2022; Condessa et al. 2024). So, here we highlight this intimate interaction with microbial communities: an adjustment on seed microbiome is found to ease germination and seed-to-seedling transition allowing establishment, while nodule microbiome is more selected to increase development and seedling-to-sapling switch. Indeed, the increased knowledge of invasive plant-microbiome interactions will contribute to a broader understanding of plant local adaptation in novel ecosystems.

Conclusions

Our study suggests that *Acacia longifolia* adapts the microbiome according to its needs, depending on the surrounding conditions. The microbiome of this invasive plant species plays a pivotal role in the plant's success across environments, aiding invasive success. Our findings suggest a trade-off between microbial diversity and functional specialization in seed microbiome, proposing that even with lower diversity, their functionality is maintained or even increased to support the plant's initial establishment. On the other hand, the nodule microbiome reflects a greater selection of microbes, each contributing to specific physiological processes necessary for growth. In nodules, apart from *Bradyrhizobium* and nitrogen fixation contribution, a diverse and richer microbial community is harbored, performing specific functions (such as defense and metabolism-related) to sustain plant development in varying and challenging habitats. The interaction between *A. longifolia* and its microbiome highlights the role of microbial partners in invasive plants' ability to adapt to different ecosystems. This is particularly relevant in Mediterranean-type ecosystems under climate change. The plants' success in invasive range can be partly attributed to these efficient and specialized microbial associations that enhance both nutrient uptake and resistance to edaphic fluctuations.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

JGJ and AA led the data collection and curation. JGJ led the formal analysis, visualization and writing – original draft. CM and HT were responsible for supervision, validation and writing – review & editing. AA was involved in validation and writing – review & editing. HT and CM was involved in funding acquisition. All authors were responsible for conceptualization and gave final approval for publication.

Author ORCIDs

Joana G. Jesus https://orcid.org/0000-0002-8007-5031
Andreia Anjos https://orcid.org/0000-0003-3987-6862
Cristina Máguas https://orcid.org/0000-0002-4396-7073
Helena Trindade https://orcid.org/0000-0002-1209-2622

Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Unraveling the microbiome dynamics of the invasive *Acacia longifolia*: a closer look on seeds and nodules

Authors: Joana G. Jesus, Andreia Anjos, Cristina Máguas, Helena Trindade

Data type: docx

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